

SEQUENCE LISTING

<110> Cox III, George Norbert
 Case, Casey Christopher
 Eisenberg, Stephen P.
 Jarvis, Eric Edward
 Spratt, Sharon Kaye
 Sangamo Biosciences, Inc.

<120> Regulation of Endogenous Gene Expression in Cells Using
 Zinc Finger Proteins

<130> 019496-002200US

<140> 09/229,037
 <141> 1999-01-12

<160> 40

<170> PatentIn Ver. 2.0

<210> 1
 <211> 25
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:exemplary motif
 of C2H2 class of zinc finger proteins (ZFP)

<220>
 <221> MOD_RES
 <222> (2)..(3)
 <223> Xaa = any amino acid

<220>
 <221> MOD_RES
 <222> (4)..(5)
 <223> Xaa = any amino acid, may be present or absent

<220>
 <221> MOD_RES
 <222> (7)..(18)
 <223> Xaa = any amino acid

<220>
 <221> MOD_RES
 <222> (20)..(22)
 <223> Xaa = any amino acid

<220>
 <221> MOD_RES
 <222> (23)..(24)
 <223> Xaa = any amino acid, may be present or absent

<400> 1
 Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1 5 10 15
 Xaa Xaa His Xaa Xaa Xaa Xaa Xaa His
 20 25

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 102020-11026860

<210> 2
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ZFP target site
 with two overlapping D-able subsites

<220>
 <221> modified_base
 <222> (1)..(2)
 <223> n = g,a,c or t

<220>
 <221> modified_base
 <222> (5)
 <223> n = g,a,c or t

<220>
 <221> modified_base
 <222> (8)
 <223> n = g,a,c or t

<220>
 <221> modified_base
 <222> (9)
 <223> n = a,c or t; if g, then position 10 cannot be g
 or t

<220>
 <221> modified_base
 <222> (10)
 <223> n = a or c; if g or t, then position 9 cannot be g

<400> 2
 nngkngknnn

10

<210> 3
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ZFP target site
 with three overlapping D-able subsites

<220>
 <221> modified_base
 <222> (1)..(2)
 <223> n = g,a,c or t

<220>
 <221> modified_base
 <222> (5)
 <223> n = g,a,c or t

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<220>
 <221> modified_base
 <222> (8)
 <223> n = g,a,c or t

<400> 3
 nngkngkngk

10

<210> 4
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 4
 Asp Gly Gly Gly Ser
 1 5

<210> 5
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 5
 Thr Gly Glu Lys Pro
 1 5

<210> 6
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 6
 Leu Arg Gln Lys Asp Gly Glu Arg Pro
 1 5

<210> 7
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 7
 Gly Gly Arg Arg
 1

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<210> 8
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 8
 Gly Gly Gly Gly Ser
 1 5

<210> 9
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 9
 Gly Gly Arg Arg Gly Gly Gly Ser
 1 5

<210> 10
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 10
 Leu Arg Gln Arg Asp Gly Glu Arg Pro
 1 5

<210> 11
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 11
 Leu Arg Gln Lys Asp Gly Gly Gly Ser Glu Arg Pro
 1 5 10

<210> 12
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

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<400> 12
 Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Gly Ser Glu Arg Pro
 1 5 10 15

<210> 13
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ZFP target site
 region surrounding initiation site of vascular
 endothelial growth factor (VEGF) gene containing
 two 9-base pair target sites

<220>
 <221> protein_bind
 <222> (4)..(12)
 <223> upstream 9-base pair ZFP VEGF1 target site

<220>
 <221> protein_bind
 <222> (14)..(22)
 <223> downstream 9-base pair ZFP VEGF3a target site

<400> 13
 agcggggagg atcgcgagg cttgg

25

<210> 14
 <211> 298
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF1 ZFP
 construct targeting upstream 9-base pair target
 site in VEGF promoter

<220>
 <221> CDS
 <222> (2)..(298)
 <223> VEGF1

<400> 14
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg cgc 97
 Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
 35 40 45

aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc cac 193
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
 50 55 60

T02020"14326860

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
 65 70 75 80

cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag aag 289
 Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
 85 90 95

ggt gga tcc
 Gly Gly Ser

298

<210> 15
 <211> 99
 <212> PRT
 <213> Artificial Sequence

<400> 15
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg
 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
 65 70 75 80

Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
 85 90 95

Gly Gly Ser

<210> 16
 <211> 298
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: VEGF3a ZFP
 construct targeting downstream 9-base pair target
 site in VEGF promoter

<220>
 <221> CDS
 <222> (2)..(298)
 <223> VEGF3a

<400> 16
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

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tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97
 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
 35 40 45

aaa cgc ttc acc cgt tgc tca aac cta cag agg cac aag cgt aca cac 193
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
 50 55 60

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
 65 70 75 80

cga agt gac gag ctg tca cga cat atc aag acc cac cag aac aag aag 289
 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
 85 90 95

ggt gga tcc
 Gly Gly Ser

298

<210> 17
 <211> 99
 <212> PRT
 <213> Artificial Sequence

<400> 17
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
 65 70 75 80

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
 85 90 95

Gly Gly Ser

<210> 18
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: VEGF DNA target
 site 1 recognition (top) strand

TO2020"4436360

<220>
 <221> protein_bind
 <222> (11)..(19)
 <223> VEGF DNA ZFP target site 1

<400> 18
 catgcatagc ggggaggatc gccatcgat

29

<210> 19
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF DNA site 1
 complementary (bottom) strand

<400> 19
 atcgatggcg atcctccccg ctatgcatg

29

<210> 20
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF DNA
 target site 3 recognition (top) strand

<220>
 <221> protein_bind
 <222> (11)..(19)
 <223> VEGF DNA ZFP target site 3

<400> 20
 catgcatatc gcggaggctt ggcacgat

29

<210> 21
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF DNA target
 site 3 complementary (bottom) strand

<400> 21
 atcgatgcca agcctccgag atatgcatg

29

<210> 22
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer SPE7

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29

26

28

26

83

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<210> 27
<211> 39
<212> DNA
<213> Artificial Sequence
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<220>
 <223> Description of Artificial Sequence:primer GB19

<400> 27 39
 gccatgccgg tacccatacc tggcaagaag aagcagcac

<210> 28
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer GB10

<400> 28 33
 cagatcggat ccacccttct tattctggtg ggt

<210> 29
 <211> 589
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:designed
 6-finger ZFP VEGF3a/1 from KpnI to BamHI

<220>
 <221> CDS
 <222> (2)..(589)
 <223> VEGF3a/1

<400> 29
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97
 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
 35 40 45

aaa cgc ttc aca cgt tcg tca aac cta cag agg cac aag cgt aca cac 193
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
 50 55 60

aca ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
 65 70 75 80

cga agt gac gag ctg tct aga cac atc aaa acc cac cag aac aag aaa 289
 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
 85 90 95

gac ggc ggt ggc agc ggc aaa aag aaa cag cac ata tgt cac atc caa 337
 Asp Gly Gly Gly Ser Gly Lys Lys Lys Gln His Ile Cys His Ile Gln
 100 105 110

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ggc tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg 385
 Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu
 115 120 125

cgc tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt 433
 Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys
 130 135 140

ggt aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc 481
 Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr
 145 150 155 160

cac acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc 529
 His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe
 165 170 175

atg cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag 577
 Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys
 180 185 190

aag ggt gga tcc 589
 Lys Gly Gly Ser
 195

<210> 30
 <211> 196
 <212> PRT
 <213> Artificial Sequence

<400> 30
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
 65 70 75 80

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
 85 90 95

Asp Gly Gly Gly Ser Gly Lys Lys Lys Gln His Ile Cys His Ile Gln
 100 105 110

Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu
 115 120 125

Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys
 130 135 140

Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr
 145 150 155 160

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His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe
 165 170 175

Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys
 180 185 190

Lys Gly Gly Ser
 195

<210> 31
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:JVF9 VEGF3a/1
 target oligonucleotide

<400> 31
 agcgagcggg gaggatcgcg gaggcttggg gcagccgggt ag 42

<210> 32
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:JVF10 VEGF3a/1
 target oligonucleotide complementary sequence

<400> 32
 cgctctaccc ggctgcccc a gcctccg cg atcctccccg ct 42

<210> 33
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer JVF24

<400> 33
 cgcggatccg cccccccgac cgatg 25

<210> 34
 <211> 62
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:downstream
 primer JVF25

<400> 34
 ccgcaagctt acttgatc gtcgtccttg tagtcgtgc cccaccgta ctggtcaatt 60

cc

62

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<210> 35
 <211> 7
 <212> PRT
 <213> Simian virus 40

<220>
 <221> PEPTIDE
 <222> (1)..(7)
 <223> SV40 large T antigen nuclear localization sequence
 (NLS)

<400> 35
 Pro Lys Lys Lys Arg Lys Val
 1 5

<210> 36
 <211> 61
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:segment from
 EcoRI to KpnI containing Kozak sequence including
 initiation codon and SV40 NLS

<400> 36
 gaattcgcta gcgccaccat ggccccaag aagaagagga aggtgggaat ccatggggta 60
 c 61

<210> 37
 <211> 187
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:segment from
 KpnI to XhoI containing BamHI site, KRAB-A box
 from KOX1, FLAG epitope and HindIII site

<400> 37
 ggtacccggg gatcccgac actggtgacc ttcaaggatg tatttgtgga cttcaccagg 60
 gaggagtgga agctgctgga cactgctcag cagatcgtgt acagaaatgt gatgctggag 120
 aactataaga acctggtttc cttgggcagc gactacaagg acgacgatga caagtaagct 180
 tctcgag 187

<210> 38
 <211> 277
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:inserted
 fragment from BamHI to HindIII sites

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<400> 38

ggatccgccc ccccgaccga tgtcagcctg ggggacgagc tccacttaga cggcgaggac 60
 gtggcgatgg cgcattgccga cgcgctagac gatttcgata tggacatgtt gggggacggg 120
 gattccccgg ggccgggatt tccccccac gactccgccc cctacggcgc tctggatatg 180
 gccgacttcg agtttgagca gatgtttacc gatgcccttg gaattgacga gtacggtggg 240
 ggagcgact acaaggacga cgatgacaag taagctt 277

<210> 39

<211> 118

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:sequence
 replacing NLS-KRAB-FLAG with NLS-FLAG only

<400> 39

gaattcgcta gcgccaccat ggcccccaag aagaagagga aggtgggaat ccatggggta 60
 cccgggggatg gatccggcag cgactacaag gacgacgatg acaagtaagc ttctcgag 118

<210> 40

<211> 204

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:insert into
 MluI/BglII sites of pGL3-Control to create
 pVFR1-4x

<400> 40

acgcgtaagc ttgctagcga gcggggagga tcgcggaggc ttggggcagc cgggtagagc 60
 gagcggggag gatcgcgag gcttggggca gccgggtaga gcgagcgggg aggatcgagg 120
 aggcttgggg cagccgggta gagcgagcgg ggaggatcgc ggaggcttgg ggcagccggg 180
 tagagcgctc agaagcttag atct 204

SCANNED, # 14